UNIVERSITY DEPARTMENT OF ZOOLOGY

RANCHI UNIVERSITY, RANCHI

SYNOPSIS FOR Ph.D

Topic: Studies on Bacterial communities associated with litchi looper, *Perixera illepidaria* (Guenèe) (Lepidoptera: Geometridae) and its parasitoids

Under the supervision of:

Dr. Seema Keshari

Assistant Professor

University Department of Zoology

Ranchi University, Ranchi

Submitted by-

Madhuri Kumari

DST- Inspire Fellow

STATE OF THE ART

Litchi (*Litchi chinensis sonn.*) is one of the most significant subtropical fruit trees of the family Sapindaceae. India is the second-largest producer of litchi in the world after China, with an area of around 74.40 thousand hectares and a total production of 483.60 thousand metric tonnes (Kumar and Kumari 2020). About 60% of India's total output of litchis is produced in the eastern states of Bihar, Jharkhand, and West Bengal. 64.42 thousand metric tonnes of litchi, or nearly 9% of the nation's total production, were produced in Jharkhand in 2021–2022. (NHB, 2021-2022).

INTERNATIONAL-

Jeremy K Nicholson *et al.*, 2012 made study on the gut microbiota that involved in the regulation of multiple host metabolic, signalling, and immune-inflammatory axes. Philipp Engel, Nancy A. Moran 2013 studied and hypothesized host diet and taxonomy structure intestinal bacterial community composition among insects. According to the finding, bees and wasps had the least abundant bacterial communities, whereas insect that consumed decaying wood had the most diverse gut flora. Host diet was an important community structuring factor. Ji-Hyun Yun *et al.*, 2014 studied and characterized the gut microbiota of 305 individuals belonging to 218 species in 21 taxonomic orders, identifying 9301 bacterial operational taxonomic units (OTUs). Gut bacterial diversity was higher in omnivorous than stenophagous insect and predominant proteobacteria and fermicutes. Yuan *et al.,* 2021examined the impact of several hosts (corn, wild oat, oilseed rape, pepper, and artificial diet) on the diversity and organization of the gut microbial community in *Spodoptera frugiperda*. The gut microbial population was found to be dominated by fermicutes and bacteriodetes. According to PCoA analysis, the gut microbial population differed considerably among the different hosts. The majority of the functional prediction categories were connected to metabolic and cellular activities, according to PICRUSt analysis. The findings demonstrated that the host species and various host treatments had an impact on the intestinal microbial population of *S. frugiperda*, which was crucial for host adaptability.

NATIONAL-

Poorani and Mohansundaram 2022 analyzed that larvae of *Perixera illepidaria* (Lepidoptera) migrate to non-host plant (banana) for pupation, and parasitism of mature larvae and pupae was observed on banana, a non-host, rather than on mango, the natural host. Mango, the natural host,

showed no signs of parasitism. And 5 pupal parasitoids were reported on *P. illepidaria* and all were collected on banana. J. Nayanathara and R. Narayana 2022 identified that production and productivity are being threatened by the lepidopteran pest complex in mango panicles. 17 specimens from seven families were identified from four randomly selected districts in Kerala, and the caterpillar complex poses a threat to the crop's reproductive phase.

REGIONAL-

Vinod Kumar *et al.*, 2014 observed *Perixera illepidaria* (Lepidoptera: Geometridae) was found on litchi trees in the India state of Bihar. The incidence of looper infested trees varied from 34.1 to 84.5%, with the mean number of larvae per 10 leaflets ranging from 0.6 to 14.7. This is the first account of *P. illepidaria* on litchi trees in India. Naaz *et al*., 2016 identified the cultivable gut bacteria associated with peach fruit fly, *Bactrocera zonata*, and evaluated their potential to attract adults. According to molecular phylogeny, Rhodococcus spp. and Microbacterium spp. belonged to the gramme positive Actinobacteria group, while *Klebsiella oxytoca* belonged to the gramme negative -proteobacteria. Adult females of *B. zonata* were more attracted to *K. oxytoca*-derived metabolites. This research might lead to the creation of a fly population control approach that targets females..

Insects are the animal clad with the greatest diversity and abundance of species worldwide (Basset *et al.*, 2012). Lepidoptera is the largest order of class insecta that includes butterflies and moths. It includes about 180,000 species of insect in which *Perixera illepidaria* is the major lepidopteran insect pest on Litchi (Kumar *et al.*, 2014) and Mango (Saumya *et al.*, 2021). One of the major factors limiting the quantity and quality of litchi is pest and disease. They indirectly reduce the yield by destroying trees. Other insect pest that affect litchi in India has been recorded as fruit and seed borers or litchi stem end borers (Singh 1975, 1978; Butani 1977; Waite & Hawang 2002; Nair & Sahoo2006). Most lepidopteran feed on plants with their piercing mouthparts.

According to studies endosymbionts present in insects include viruses, bacteria, fungi, protozoa, nematodes and multicellular parasites (Buchner 1965; Douglas 1998; Moran *et al.* 2005). In insects, the bacterial communities mostly found in the gut, hemocoel, and mycetomes and within the cells. Out of these bacteria, major population cannot be cultured on the growth media. Thus both culture dependent and culture independent approaches were used for analyzing the gut

bacteria of insect. The bacteria play a significant role in various metabolic functions of insects, i.e nutrition, digestion, protection, and detoxification of insecticides, severe heat tolerance and a source of cues and signals synthesis (Dillon and Dillon 2004). They also provide digestive enzymes or vitamins lacking in fruit tissues of their host and improve digestion efficiency which is ultimately manifested in the development, fecundity, and survivability of their hosts (Nakabachi and Ishikawa 1999). Understanding the gut bacteria of the insects and their potential role in larval physiology could give rise to new targets for eco friendly pest management. The level of manmade environmental change is constantly growing, which might be the cause of an outbreak. Schreiner & Nafus (1992) documented population changes in 4 Lepidoptera species, including the widespread distribution of *P. illepidaria*.

Due to their diversity, ecological significance, and impact on agriculture, human health, and natural resources, insects play a significant role in society. Additionally, it establishes the biological framework for each terrestrial ecosystem. They maintain soil structure and fertility, cycle nutrients, pollinate plants, disperse seeds and provide as a significant source of food for other species (Geoffery G. E. Scudder *et al,*2017) but many insects are considered to be the pests which reduce the plant density, lower production capacity, reduce the yield or quantity of agricultural products, etc. which affect our economy.

So my research work is about Lepidoptera, *P. illepideria* (litchi looper) of the family Geometridae which consumes fragile plant components. *P. illepidaria* is a significant pest of litchi and has also been reported a pest of mango. It is disturbed in the north eastern Himalayas, Hong Kong, Sundaland, the Philippines and Thailand. In India, *P. illepidaria* was initially identified as an epidemic pest on litchi during the 2011–12 growing season in Bihar. It is identified as a major pest concern on commercial cultivars of mango in the southern Indian states of Andhra Pradesh, Karnataka and Tamil Nadu in 2020-21.

The current study aims to identify the cultivable and non-cultivable bacteria found in insect guts, characterize them using the 16S rRNA gene and metagenomic sequencing, and describe the major adult parasitoids' associated bacterial communities with host-specific populations of litchi loopers.

PROBLEMS TO BE INVESTIGATED

1. Bacterial communities variation across different developmental stages of litchi looper, *P. illepidaria.*
2. Interaction effect of bacterial communities among pest (*P. illepidaria*) and its parasitoids.

OBJECTIVES

1. To isolate and characterize the cultivable/ non cultivable bacterial communities associated with different developmental stage of litchi looper, *P. illepidaria.*
2. To characterize the bacterial communities of major adult parasitoids associated with different litchi looper, *P. illepidaria* host specific populations.

MOTIVATION

Types of the bacterial community, their functions, variation in numbers and types of gut bacteria in the different development stage of the host could help in the integrated pest management to overcome the pest problem.

HYPOTHESIS

Litchi looper *P. illepidaria* is a significant pest of litchi, mango etc. and it is very essential to know it's fitness on different host and environment. It is hypothesized that this understanding will help to design microbiota-based management strategies. The study also helps to understand the pest-parasitoids fitness relationship. The variation of bacterial communities can be understood through different diversity indices.

RESEARCH DESIGN AND METHODOLOGY

A. <u>COLLECTION AND INITIATION OF THE INSECT CULTURE</u>

1. Different stages of *P. illepidaria* will be collected from the Research Farm of ICAR-RCER, Ranchi and nearby location of Ranchi.
2. These will be identified based on the morphological descriptions given by (Kumar *et al.*, 2014).

B. DISSECTION AND ISOLATION OF GUT BACTERIA

1. Different stages of *P. illepidaria* will be anesthetized and surface sterilized.
2. Dissected aseptically to remove gut under laminar air flow.
3. Gut contents will be streaked on different suitable media (Nutrient Agar, Peptone Yeast Extract Agar,) for bacterial growth at 30±1°C for 48-72 h.
4. A single colony will be separated and streaked onto suitable media (NA, PYEA etc).
5. Predominated bacteria will be isolated through sub culturing and maintained on respective medium slants or plates at 4 -8 °C.

C. BIOLOGICAL AND MORPHOLOGICAL CHARACTERIZATION

1. Morphological, Cultural, Biochemical characterization of the pure culture will be done by standard techniques.
2. Isolates characteristics will be compared with Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 2000).

D. MOLECULAR CHARACTERIZATION

1. Total genomic DNA of each bacterial isolate will be extracted following the method of (Naaz *et al.,* 2016) with some minor modification.
2. PCR amplification of 16S rRNA genes through universal bacterial primers.
3. PCR products will be sequenced and sequences will be identified using online NCBI Blast program.
4. Bacterial isolates sequences will align and phylogenetic tree will be constructed using MEGA ver. software or by using other bioinformatics tools.
5. Metagenomic sequencing is used for identifying non-cultivable bacteria in complex bacterial communities.

E. CHARACTERIZATION OF THE BACTERIAL COMMUNITIES ASSOCIATED WITH STAGE SPECIFIC PARASITOIDS

1. Collection of field population of immature stage and reared in laboratory condition.
2. Host and parasitoids of stage specific emergence will be monitored daily
3. Parasitoids that emerged during the collection will be reared and kept in glass jars, and will be feed with honey.
4. Same procedure of above will be followed for DNA extraction and furthers analyses.

TOOLS TO BE USED

1. Laminar air flow
2. B.O.D incubator
3. Autoclave
4. Hot air oven
5. Water bath
6. Centrifuge
7. PCR and its related chemicals and tools, etc.
8. Other tools as per the need of work.

SIGNIFICANCE OF STUDY

1. Contributes to the study would provide greater understanding of bacterial communities of stage of *P. illepidaria*.
2. Stages, age, and host-associated bacterial diversity-related information will be provided which are important to their survivability and fitness.
3. Information will be used to develop eco-friendly management strategies against this devastating pest.
4. Information will be useful to an understanding of evolutionary knowledge on species establishment.

FUTURE ASPECTS

The future prospect of the present work is to develop comparative studies among of loppers on economically important fruit plants. Researchers may test the fitness of pests with different types of host plants to calculate the future threat.

REFERENCES

1. Abdelfattah A, Malacrinò A, Wisniewski M, Cacciola SO, Schena L (2017) Metabarcoding: a powerful tool to investigate microbial communities and shape future plant protection strategies. Biol Control 120:1–10.
2. AE Douglas (1998). Nutrional interactions in insects- Microbial symbioses: Aphids and their symbiotic bacteria *Buchnera*. *Annual review of entomology* 43(1), 17-37, 1998.
3. Atlas, R. M. (1984). Diversity of microbial communities. In Advances in microbial ecology (pp. 1-47). Springer, Boston, MA.
4. Basset, Y., Cizek, L., Cuènoud, P., Didham, R. K., Guilhaumon, F., Missa, O., & Tishechkin, A. K. (2012) Arthropod diversity in tropical forest. Science, 338(6113), 1481-1484.
5. Buchner, P. (1965) Endosymbioses of Animals with Plant Micro-Organisms. John Wiley & Sons, Chichester.
6. Dillon, R. J., & Dillon, V. M. (2004). The gut bacteria of insects: nonpathogenic interactions. *Annual Review of Entomology*, 49(1), 71-92.
7. Engel, P., & Moran, N. A. (2013, September). The gut microbiota of insects–diversity in structure and function. *FEMS Microbiology Reviews*, *37*(5), 699–735. https://doi.org/10.1111/1574-6976.12025
8. Holt, J. G. (1977). The shorter Bergey's manual of determinative bacteriology. The shorter Bergey's manual of determinative bacteriology. 8th edition.
9. Ilse H. Schreiner, Donald M. Nafus, Changes in a Moth Community Mediated by Biological Control of the Dominant Species, Environmental Entomology, Volume 21, Issue 3, 1 June 1992, Pages 664–668, https://doi.org/10.1093/ee/21.3.664
10. J. Nayanathara and R. Narayana (2022). Caterpillar Complex: A Rise in Threat to Mango Panicle. Indian Journal of Ecology (2022) 49(1):252-256 DOI: https://doi.org/10.55362/ije/2022/3512.

11. Jankiraman Poorani, Arumugam Mohanasundaram (June 2022) Larvae of *Perixera illepidaria* (Guenée) (Lepidoptera: Geometridae), an emerging pest of mango, migrate to banana for pupation and to meet their nemesis. Current Science 122(12):1367-1370.
12. Janmejay Kumar and Nikky Kumari (2020) Growth and Stability of Litchi Production In Muzaffarpur District of Bihar, India 9(5):708-719 DOI: https://doi.org/10.20546/ijcmas.2020.905.079.
13. Lv, D., Liu, X., Dong, Y., Yan, Z., Zhang, X., Wang, P., Yuan, X., & Li, Y. (2021, October 19). Comparison of Gut Bacterial Communities of Fall Armyworm (Spodoptera frugiperda) Reared on Different Host Plants. *International Journal of Molecular Sciences*, *22*(20), 11266. https://doi.org/10.3390/ijms222011266.
14. Naaz, N., Choudhary, J. S., Prabhakar, C. S., & Maurya, S. (2016). Identification and evaluation of cultivable gut bacteria associated with peach fruit fly, *Bactrocera zonata* (Diptera:Tephritidae) *Phytoparasitic*a, 44(2),165-176.
15. Nakabachi, A. & Ishikawa, H. (1999) Provision of riboflavin to the host aphid, Acyrthosiphon pisum, by endosymbiotic bacteria, Buchnera. Journal of Insect Physiology, 45, 1–6.
16. Nancy A Moran, Patrick H Degnan, Scott R Santos, Helen E Dunbar & Howard Ochman (2005). The players in a mutualistic symbiosis: insect, bacteria, viruses, and virulence genes. Proceedings of the National Academy of Sciences 102 (47), 16919-16926, 2005
17. NHB- National horticulture board, Ministry of Agri. & Farmers welfare; Govt. of India report 2021-2022.
18. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. Host-gut microbiota metabolic interactions. Science. 2012 Jun 8;336(6086):1262-7. doi: 10.1126/science.1223813. Epub 2012 Jun 6. PMID: 22674330.
19. Robert I. Grahama,, Shujing Raoc, Steven M. Sait d, Houssam Attoui , Peter P.C. Mertens, Rosemary S. Hails , Robert D. Posseea (2008). Sequence analysis of a reovirus isolated from the winter moth Operophtera brumata (Lepidoptera: Geometridae) and its parasitoid wasp Phobocampe tempestiva (Hymenoptera: Ichneumonidae). *Elsevier* Virus Research 135(2008)42–47.

20. Soumya B. R., Abraham Varghese, (2021) Threat of loopers (Perixera illepidaria) on mango in different parts of south india. (IE Blog) Insect Environment https://insectenvironment.com
21. Soumya B.R., Rashmi, Verghese Abraham (2021) Bioecology of the mango inflorescence caterpillar, Perixera illepidaria Guenee and record of its feeding on mango fruits. Pest management in Horticulture, Article DOI 10.5958/0974-4541.2021.00005.9.
22. Vinod Kumar, P.Venkata Rami Reddy, Ajit Kumar Dubedi Anal, Vishal Nath (2014). Outbreak of the Looper, *Perixera illepidaria* (lepidoptera: Geometridae) on Litchi, *Litchi Chinesis* (Sapindales: Sapindaceae) - A new pest record from India. Florida Entemologist, 97(1):22-29(2014).
23. Yun, J. H., Roh, S. W., Whon, T. W., Jung, M. J., Kim, M. S., Park, D. S., & Kim, J. Y. (2014). Insect gut bacterial diversity is determined by environmental habitat, diet, developmental stage, and phylogeny of a host. *Applied and Environmental Microbiology*, 80(17), 5254-5264.